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# Association of Circulating Markers With Outcome Parameters in the Bevacizumab and Ranibizumab in Diabetic Macular Edema Trial

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See the appendix for the members of the BRDME research group.

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**PURPOSE.** The purpose of this study was to evaluate selected candidate biomarkers as potential markers for patients with diabetic macular edema (DME) who receive antivasculature endothelial growth factor (VEGF) therapy

**METHODS.** Selected biomarkers included blood levels of messenger RNA (mRNA) of retinoschisin, RPE65, rhodopsin, and endothelial progenitor cell markers CD34 and CD133. Blood samples were obtained from 89 patients with DME according to the study protocol of the Bevacizumab and Ranibizumab in Diabetic Macular Edema (BRDME) study. During each monthly visit, patients underwent optical coherence tomography scanning and visual acuity was measured. Anti-VEGF injections were administered at fixed monthly intervals over 6 months. Analyses of covariance using simplified and linear mixed models were used to examine the correlations between candidate markers and changes in visual acuity and central subfield thickness.

**RESULTS.** Plasma mRNA levels of retinoschisin were negatively associated with visual acuity, and plasma mRNA levels of rhodopsin were positively associated with visual acuity in patients with DME ( $P < 0.01$  and  $P < 0.05$ , respectively). In addition, changes in central subfield thickness between baseline and months 1, 2, and 3 during anti-VEGF treatment were associated with mRNA levels of retinoschisin, rhodopsin, and the ratio of retinoschisin-to-rhodopsin ( $P < 0.01$ , all).

**CONCLUSIONS.** This prospective, multicenter study found that circulating mRNA levels of retinoschisin and rhodopsin are associated with visual acuity and changes in central subfield thickness during anti-VEGF therapy in patients with DME. (ClinicalTrials.gov number: NCT01635790.)

**Keywords:** biomarker, diabetic macular edema, retinoschisin, rhodopsin, visual acuity

Diabetic retinopathy (DR) is a common and specific microvascular complication of diabetes mellitus that may progress to vision-threatening retinopathy, including diabetic macular edema (DME). The prevalence of DME is increasing as the prevalence of diabetes is increasing sharply.<sup>1</sup> The foundation for reduction of the risk of DR progression includes optimal control of blood glucose, blood pressure, and possibly blood lipids. Despite having good control of these systemic risk factors, a significant proportion of patients still develop vision-threatening DME.<sup>2</sup> The Diabetes Control and Complications Trial showed that the levels of HbA<sub>1c</sub> explained only approximately 15% of the total variation in the risk of progression of DR.<sup>3</sup> Thus, new biomarkers for disease progression are needed. Biomarkers for DME may contribute to diagnosis, understanding of pathogenesis, response to treatment, and further development of alternative treatment strategies targeted at newly stratified

patient groups. However, presently, biomarkers for these DME parameters have not been established.

In recent years, the understanding of the complex processes involved in DR has grown rapidly and has provided tools with which to select candidate biomarkers based on pathogenic mechanisms and structural damage. Endothelial progenitor cells (EPCs) are a low-frequency population of circulating cells that are recruited to sites of vessel damage and tissue ischemia, where they promote vascular healing and reperfusion.<sup>4</sup> A growing body of evidence suggests DR development is associated with altered numbers of EPCs. In fact, some EPC subtypes, including CD34<sup>+</sup> and CD133<sup>+</sup> cells, may be directly involved in the pathogenesis of DR and may serve as biomarkers for DR disease progression.<sup>5–8</sup> Other candidate biomarkers may be related to structural retinal damage in DR. These candidate biomarkers include mRNA levels of rhodopsin,



RPE65, and retinoschisin and have been independently and significantly associated with disease progression of DR.<sup>9-14</sup>

Using blood samples collected in the Bevacizumab to Ranibizumab in patients with Diabetic Macular Edema (BRDME) study, in which the effectiveness and costs of bevacizumab and ranibizumab are compared in patients with DME,<sup>15</sup> we explored the most strongly associated leads in DR biomarker research. In this prospective, multicenter study, we evaluated the diagnostic and prognostic value of mRNA of EPC markers CD34, CD133, and plasma mRNA of rhodopsin, RPE65, and retinoschisin in patients with DME. Our hypothesis was that individual selected candidate biomarkers or combinations of these biomarkers might serve as useful markers for outcome in patients with DME.

## MATERIALS AND METHODS

### Patient Characteristics

Blood samples of DME patients included in the ongoing BRDME study<sup>15</sup> were analyzed. The BRDME study was a large clinical trial in which patients with DME receive monthly anti-vascular endothelial growth factor (anti-VEGF) injections during 6 months in 7 university medical centers in the Netherlands (Dutch Trial Register NTR3247 and ClinicalTrials.gov NCT01635790). Study participants who had completed the BRDME study by July 2015 were included in the study. The BRDME study was approved by the Medical Ethical Review Committee of the Academic Medical Center Amsterdam. The participation of the other centers was reviewed at each center according to Dutch law.

The study protocol of the BRDME study was published previously.<sup>15</sup> The most relevant inclusion criteria for DME patients were (1) clinically significant macular edema as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS); (2) central macula thickness of  $>325\ \mu\text{m}$  as documented using optical coherence tomography (OCT); (3) no previous intravitreal anti-VEGF or triamcinolone injections within 3 months prior to randomization; (4) no previous macular focal laser therapy within 3 months of commencement of the study period; and (5) age  $>18$  years old. The most relevant exclusion criteria were (1) presence of ocular disease other than DR and cataract; (2) uncontrolled glaucoma; (3) intraocular surgery, injection, or laser photocoagulation within 3 months of commencement of the study period; and (4) active untreated proliferative DR. The ETDRS severity scale was used to grade DR.

At the screening visit, the patient signed an informed consent form, and the medical and ophthalmic history was taken. Within 14 days after randomization, the patient received the first intravitreal injection of the study drug. During each visit, vital signs (heart rate and blood pressure), concomitant medication, and adverse events were recorded. Best corrected visual acuity letter score (BCVA) was assessed, and an OCT examination was performed by certified personnel prior to the intravitreal injection. The interval between visits was  $30 \pm 7$  days. It has been reported that retinal thickness measurements may demonstrate variation over the course of the day. To account for circadian fluctuations, all participants were examined before each injection between 10 AM and 1 PM.

### Donor Eyes

Eyes from nondiabetic donors were used to compare expression levels of retinal and plasma mRNA of rhodopsin and retinoschisin with expression levels in retina and retinal pigment epithelium (RPE); eyes were provided by the Corneabank Beverwijk (<http://www.eurotissuebank.nl/>

comeabank/) the Netherlands. In the Netherlands, the use of donor material is provided for by a law named “Wet op Orgaan Donatie” (WOD, Dutch Organ Donation Act). Following this law, donors provide written informed consent for donation, with an opt-out choice, for the use of leftover material for related scientific research. Specific requirements for the use for scientific research of leftover material originating from corneal grafting have been described in an additional document formulated by the Ministry of Health, Welfare, and Sport and The Bio Implant Services (BIS) Foundation (Eurotransplant; Leiden, July 21, 1995; 6714.ht). The current research was performed in accordance with all requirements stated in the WOD and the concerning document. Approval of the local medical ethics committee was not required as data were analyzed anonymously.

### Sample Collection and RNA Isolation

At the screening visit, peripheral venous blood (2.5 mL) was drawn directly into PAXgene blood RNA tubes specially designed for the collection and stabilization of RNA from whole blood (PreAnalytiX; Qiagen BD, Valencia, CA, USA). Whole-blood RNA was extracted using the PAXgene blood RNA kit, including treatment with DNase I to prevent genomic DNA contamination, strictly following the manufacturer's instructions (Qiagen). Total RNA was isolated from dissected retinas (pooled from 3 nondiabetic eyes) and human donor RPE cells<sup>16</sup> (pooled from 5 nondiabetic eyes) in TRIzol reagent (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. Total RNA was dissolved in 50  $\mu\text{L}$  of RNase-free water and measured using a NanoDrop instrument (model ND1000 spectrophotometer; NanoDrop Technologies, Wilmington, DE, USA). All samples had an optical density (OD) of  $\text{OD}_{260}:\text{OD}_{280}$  ratio  $>1.90$ . Extracted RNA was stored at  $-80^\circ\text{C}$  until required for cDNA synthesis.

### Complementary DNA (cDNA) Synthesis and Real-Time PCR

A 1- $\mu\text{g}$  aliquot of total RNA was reverse transcribed into first-strand cDNA using the Maxima first-strand cDNA synthesis kit (Thermo Scientific, Roskilde, Denmark). Real-time quantitative PCR was performed using a CFX96 real time (RT)-PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). Primer details for the RT-PCR are listed in Table 1. Specificity of the primers was confirmed using the National Center for Biotechnology Information basic local alignment search tool (BLAST). The presence of a single PCR product was verified by both the presence of a single melting temperature peak and the detection of a single band of the expected size on a 3% agarose gel. Nontemplate controls were included to verify the method and the specificity of the primers. For each primer set, a master mixture was prepared consisting of  $1\times$  iQ SYBR Green Supermix (Bio-Rad) and 2 pmol of primers completed with RNase-free water. One microliter of cDNA (diluted 1:10) in 19  $\mu\text{L}$  of Master Mix was amplified using the following PCR protocol:  $50^\circ\text{C}$  for 2 minutes and  $95^\circ\text{C}$  for 5 minutes, followed by 40 cycles of  $95^\circ\text{C}$  for 10 seconds and  $60^\circ\text{C}$  for 45 seconds, followed by  $95^\circ\text{C}$  for 1 minute, and a melting program ( $60^\circ\text{C}$ – $95^\circ\text{C}$ ). Relative gene expression was calculated by using the equation  $[r = 2^{-C_t} \times 10^{12}]$ , where  $C_t$  is the cycle threshold for the gene as determined during RT-PCR. Each sample was measured 4 or 5 times in independent runs.

### Statistical Analysis

An analysis of covariance was conducted using a simplified model analysis to assess the relationship between baseline

TABLE 1. Characteristics, Detection Rates, and Primer Details of Selected Candidate Biomarkers

Gene	Description	GenBank Accession Number	Forward Primer	Reverse Primer	% Reliable Measurements
<i>CD34</i>	EPCs (CD34 <sup>+</sup> )	NM_001025109	GGAGCAGGCTGATGCTGATG	ATCCCCAGCTTTTTCAGGTCAGAT	52
<i>PROM1</i>	EPCs (CD133 <sup>+</sup> )	NM_006017.2	CGGAGGACGTGTACGATGATGTTG	GATGGGCTTGTCTATACACAGGATTGTG	Not detected
<i>RHO</i>	Rhodopsin	U49742	AAGCCTCTTGCCCTTCCAGTTCC	CCGTCTTGGACACCGGTAGCAGA	98
<i>RPE65</i>	RPE65	NM_00329	GATGCCCTTGGAAAGAAAGATGATGGTG	TCCTTGGCATTCAGAAATCAGGAGAT	Not detected
<i>RS1</i>	Retinoschisin	NM_000330	AACCCGGTCTTCTATGGCAACTC	AGGCAGGCATCAGGCCACACTT	89

Primer details, gene nomenclature, GenBank accession codes, primer sequences, and predicted sizes and melting temperatures (T<sub>m</sub>) of the amplified product for quantitative PCR. The percentage of reliable measurements in the cohort is indicated. EPCs, endothelial progenitor cells; PROM1, prominin 1; RHO, rhodopsin; RS1, retinoschisin 1; RPE65, retinal pigment epithelium-specific protein, 65 kDa.

TABLE 2. Study Population and Baseline Ocular Characteristics of All Patients

Characteristic	Value(s)
Participants, <i>n</i>	89
Age, y	63 ± 11
Sex	
% Males	63.6
% Females	37.4
Duration of DM, y	16 ± 11
Race/ethnicity	
% White	88
% Nonwhite	12
Smoking	
% Smokers	7
% Nonsmokers	93
Mean ± SD BMI	29.6 ± 5.3
Mean ± SD MAP	100.6 ± 10.8
Ocular characteristics	
Eyes, <i>n</i>	89
Mean ± SD VA, letter score	69 ± 9
Eye	
% Right	51.5
% Left	48.5
Mean ± SD CST, μm	456 ± 97

BMI, body mass index; CST, central subfield thickness; DM, diabetes mellitus; MAP, mean arterial pressure; SD, standard deviation; VA, visual acuity.

visual acuity and circulating markers. Another analysis of covariance was performed using linear mixed models to assess the strength of the relationship between each circulating marker on change in visual acuity and central subfield thickness. These linear mixed models used repeated measures and autoregressive covariance structures to account for correlations among eyes from individual patients in the study. In these exploratory analyses, point estimates of the data and 95% confidence intervals for association were calculated. The correlations between retinoschisin and rhodopsin mRNA levels were expressed as Kendall's tau coefficient. A *P* value <0.05 was considered statistically significant.

Central subfield thickness was calculated from the OCT scans obtained at the BRDME baseline and exit visits. After subtraction of a threshold value of 250 μm (taken as the value for normal central subfield thickness), relative changes in central subfield thickness compared to baseline were calculated for each time point. We used relative changes in central subfield thickness because this value was believed to be a better indicator of proportionate treatment effects across a wide variety of baseline retinal thicknesses than the absolute change (in micrometers). For each time point, a decrease of >10% in central subfield thickness compared to baseline was defined as “responders,” and a decrease of ≤10% or an increase in central subfield thickness was defined as “nonresponders.” A sample size of 37 in each group had 80% power to detect a difference in means of 50 μm, assuming a common standard deviation of central subfield thickness of ±75 μm in both groups, using a two-group Student *t*-test with a significance level of 0.05. For responder and nonresponder analyses, both retinoschisin and rhodopsin mRNA levels were log10 transformed to obtain a normal distribution. Responders and nonresponders were compared with an unpaired Student *t*-test for log10 transformed retinoschisin and rhodopsin mRNA data and with an unpaired *t*-test with Welch's correction for



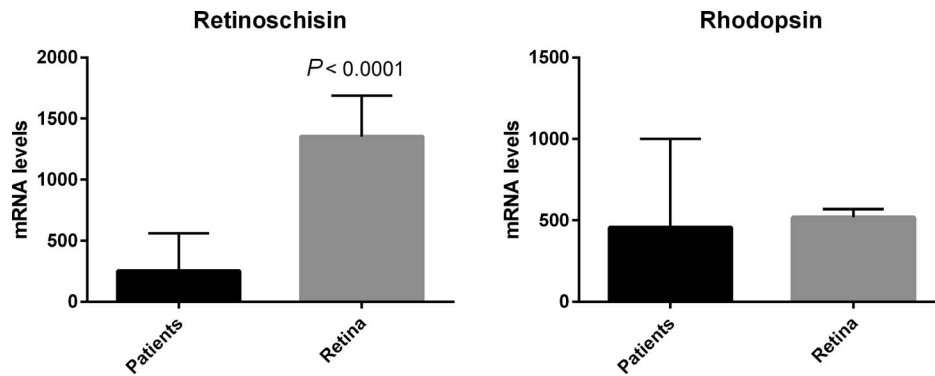


FIGURE 1. Relative mRNA levels in plasma of patients with diabetic retinopathy ( $n = 89$ ) for retinoschisin and rhodopsin are compared to those in donor retina. Values are mean  $\pm$  SD.

$\log_{10}(\text{retinoschisin mRNA})$ -to- $\log_{10}(\text{rhodopsin mRNA})$  ratios using Prism version 6 software (GraphPad Software, Inc., La Jolla, CA, USA).

## RESULTS

### Population Characteristics and Selected Biomarkers

The population and study eye characteristics of the 89 enrolled patients are summarized in Table 2. Participants had a mean  $\pm$  SD age of  $63 \pm 11$  years old, 64% were male, and 7% were smokers. The mean body mass index was  $29.6 \pm 5.3$  kg/m<sup>2</sup>, mean arterial pressure was  $100 \pm 11$  mmHg, and duration of diabetes was  $16 \pm 11$  years. The mean visual acuity letter score at baseline was  $69 \pm 9$  ETDRS letters (Snellen equivalent, approximately 20/40), and the mean central subfield thickness was  $456 \pm 97$   $\mu$ m. CD34 mRNA, used in our study as a marker of EPCs, was detected in 52% of the population cohort. The detection rates of mRNA levels of rhodopsin and retinoschisin were 89% and 98%, respectively. RPE65 and EPC CD133 mRNA levels were not detected in the blood samples of our cohort (Table 1). To the best of our knowledge, retinoschisin and rhodopsin are locally produced retina-specific proteins. Therefore, we expected that mRNA levels of rhodopsin and retinoschisin would be higher in retina than in plasma. We observed that the relative mRNA levels of rhodopsin were similar in plasma of patients with DME and donor retinas ( $P = 0.14$ ). Circulating mRNA levels of retinoschisin were 5-fold lower in plasma of patients with DME than in donor retina ( $P < 0.001$ ) (Fig. 1). No expression of mRNA retinoschisin and rhodopsin were found in RPE cells.

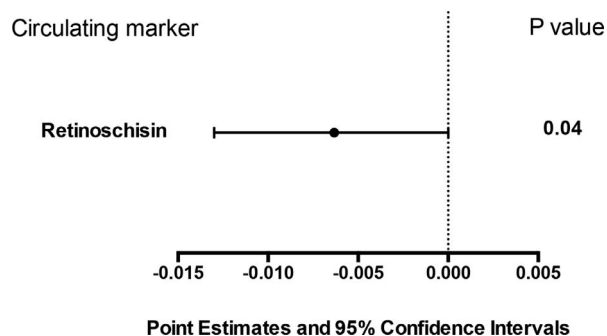


FIGURE 2. Forest plot demonstrates the association between mRNA levels of retinoschisin and BCVA at baseline. Solid boxes indicate point estimates and error bars: 95% confidence intervals.

### Association of Biomarkers With Visual Acuity

In linear regression analysis, mRNA levels of retinoschisin were statistically significantly associated with visual acuity at baseline ( $P = 0.04$ ) (Fig. 2). Unadjusted univariate analysis revealed no significant relationship between mRNA levels of rhodopsin and visual acuity. There was a strong correlation between retinoschisin and rhodopsin mRNA levels ( $\tau = 0.789$ ;  $P < 0.001$ ). In multivariate linear regression analysis, there was a statistically significant positive association between circulating mRNA levels of rhodopsin and baseline visual acuity, and mRNA levels of retinoschisin remained significantly negatively associated with visual acuity ( $P = 0.04$  and  $P < 0.01$ , respectively) (Fig. 3). There were no significant associations among mRNA levels of retinoschisin and rhodopsin or the ratio of retinoschisin-to-rhodopsin and change in visual acuity.

### Association of Retinoschisin and Rhodopsin and Outcome in DME

When changes in central subfield thicknesses were assessed, multivariate linear regression analysis revealed a significant association of change in central subfield thickness between baseline and months 1, 2, and 3 and levels of plasma retinoschisin and rhodopsin mRNA ( $P < 0.05$ , all) (Fig. 4). Central subfield thickness at baseline was included in all multivariate models as a parameter of a priori interest with regard to change in central subfield thickness. These results were further analyzed by evaluating the ratio of mRNA levels of retinoschisin and rhodopsin. Because mRNA levels of retino-

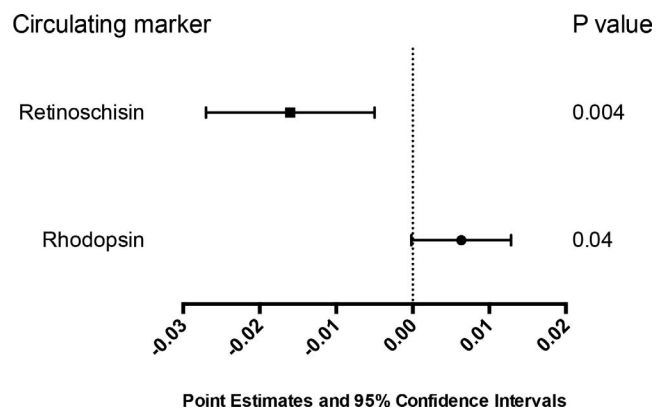


FIGURE 3. Forest plot demonstrates point estimates and 95% confidence intervals for association between baseline BCVA and mRNA levels of rhodopsin and retinoschisin.

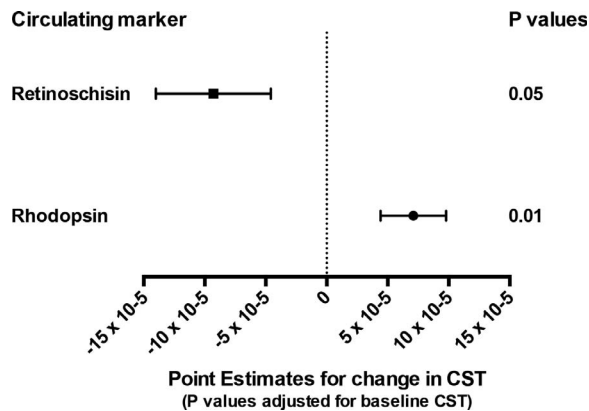


FIGURE 4. Forest plot shows the association between change in CST between baseline and month 3 and plasma retinoschisin and rhodopsin mRNA. *P* values were adjusted for baseline CST. *Squares* represent point estimates; *limit lines*, 95% confidence interval. CST, central subfield thickness.

schisin decreased with increasing BCVA, whereas mRNA levels of rhodopsin increased, combining mRNA levels of retinoschisin and rhodopsin by calculating the ratio of mRNA levels of retinoschisin-to-rhodopsin may improve the diagnostic efficiency. The ratio of mRNA levels of retinoschisin to those of rhodopsin was found to be statistically significantly different between responders and nonresponders after months 1 and 2 ( $P = 0.01$ , all) (Fig. 5). Multivariate analysis showed that the ratio of mRNA levels of retinoschisin to those of rhodopsin was also associated with change in central subfield thickness between baseline and months 1, 2, and 3 ( $P < 0.01$ , all) (Fig. 6). However, there were no significant associations between circulating markers and change in central subfield thickness between baseline and month 6 (Fig. 7). In addition, we found no significant associations among circulating mRNA levels of retinoschisin, rhodopsin, or retinoschisin-to-rhodopsin ratio with change in visual acuity during the 6-month study period. Thus, these results may indicate that plasma retinoschisin, rhodopsin, and the retinoschisin-to-rhodopsin mRNA ratio are associated with changes in central subfield thickness between baseline and months 1 to 3.

## DISCUSSION

The present study found that plasma mRNA levels of retinoschisin are significantly negatively associated with visual acuity and that plasma mRNA levels of rhodopsin are significantly positively associated with visual acuity in DME patients. In addition, multivariate linear regression analysis suggested that mRNA levels of retinoschisin, rhodopsin, and the ratio of retinoschisin-to-rhodopsin are associated with changes in central subfield thickness between baseline and months 1, 2, and 3 during intravitreal treatment with anti-VEGF.

Previous reports indicated that circulating retina-specific mRNA, including retinoschisin, rhodopsin, and RPE65 mRNA, may be useful in assessing the progression of DR.<sup>9,10,12,13</sup> We found that mRNA levels of retinoschisin and rhodopsin are associated with changes in central subfield thickness for up to 3 months after initiation of anti-VEGF treatment. Although not all their functions are known, rhodopsin and retinoschisin may play an active role in maintaining retinal integrity.<sup>18–20</sup> These roles may involve active processes which are regularly controlled and corrected, and therefore mRNA levels measured at baseline do not necessarily relate to local effects of damage in the retina 3 months after blood sample collection. In addition, we found a statistically significant association between visual acuity and plasma mRNA retinoschisin and rhodopsin in DME patients. However, it is not clear how mRNA levels of a protein are related to the pathogenesis of a disease, as the relationship between protein levels and mRNA levels is not consistent. For example, increased mRNA levels of a protein may be indicative of damage as well as repair, and changes in gene expression level are frequently not reflected at the protein level.<sup>17</sup>

Retinoschisin is a 24-kDa cell adhesive protein that is primarily expressed in rod and cone photoreceptors and bipolar cells. It is generally thought to play an essential role in maintaining the structural integrity of the outer plexiform layer, inner nuclear layer, photoreceptor homeostasis, and preservation of synaptic structures. Knockout mouse models devoid of retinoschisin have a disorganized retina, gaps between bipolar cells, cystic cavities, and loss of the b-wave in the electroretinogram.<sup>18</sup> Similarly, in young human males, loss of retinoschisin function due to mutations in the X-linked retinoschisis gene leads to progressive loss of central vision in

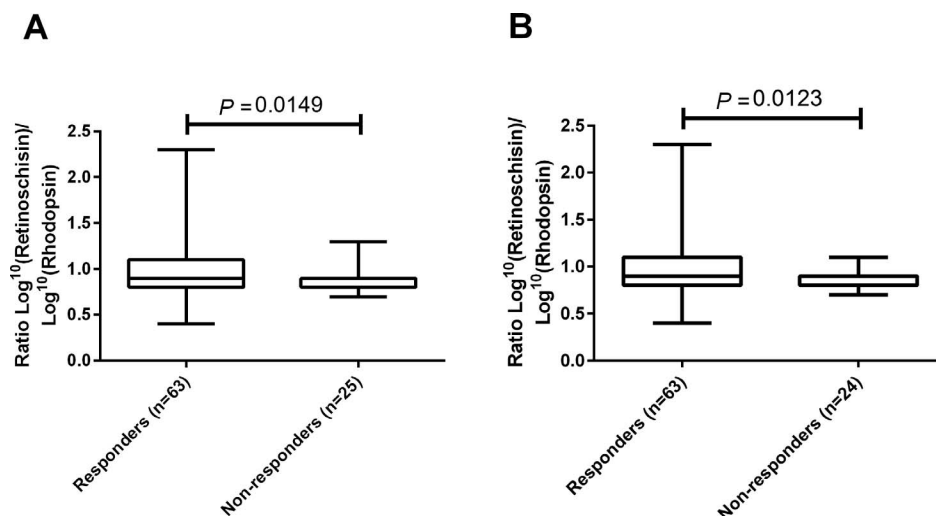


FIGURE 5. Correlation between the ratio of mRNA levels of retinoschisin and rhodopsin in responders to those of nonresponders and baseline at 1 month (A) and at 2 months (B). Responders are defined as patients showing  $>10\%$  decrease in CST and nonresponders as patients demonstrating  $\leq 10\%$  decrease or increase in CST. The ratio was taken from  $\log_{10}$  normalized values of mRNA levels of retinoschisin and rhodopsin. *Boxplots* with whiskers from minimum to maximum are shown; *central line* represents the median. CST, central subfield thickness.

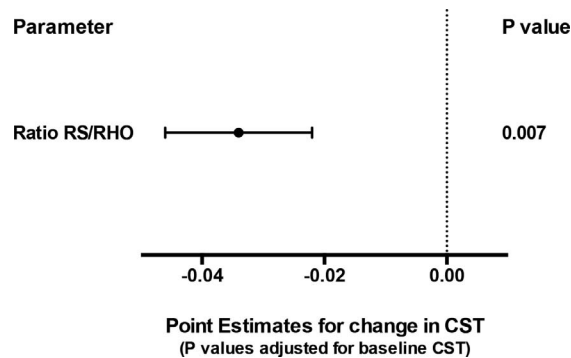


FIGURE 6. Forest plots show point estimates and 95% confidence intervals for association between change in CST between baseline and month 3 and the ratio of mRNA levels of retinoschisin and rhodopsin. *P* values were adjusted for baseline CST. CST, central subfield thickness.

the first decade of life and is characterized by splitting of the retina and cystic cavities radiating from the central retina.<sup>19</sup> Previously it was found that retinoschisin mRNA in diabetic persons without retinopathy was significantly higher than the levels seen in healthy controls.<sup>9</sup> Our study showed that retinoschisin mRNA is present in the circulation at surprisingly high levels and that these levels may be even higher in patients with DME and reduced visual acuity. The exact mechanism of these observations is unclear but may be related to upregulation of retinoschisin transcription, increased secretion of mRNA, and more extensive damage of the retina in patients with DME and reduced visual acuity.

In contrast, mRNA levels of rhodopsin were associated with better visual acuity in patients with DME. Rhodopsin is the G protein-coupled light receptor in rod photoreceptor cells and is critical for rod photoreceptor cells functionality. Dysfunction of rhodopsin by various mechanisms can cause a variety of human retinal diseases, including Leber congenital amaurosis and retinitis pigmentosa.<sup>20</sup> Our finding may be related to higher amounts of active rod cells in DME patients with better visual acuity and increased levels of mRNA rhodopsin to increase chances of rod cell survival. Subsequently, the mRNA may be released across the blood-retina barrier through openings of endothelial intercellular junctions and endothelial

caveolar transcellular transport and then could be detected in the circulation. However, the question remains as to why circulating levels of mRNA retinoschisin and rhodopsin are associated with visual acuity in patients with DME and whether these mRNAs are general markers of visual acuity or specific for patients with DME. In addition, it is unknown how these mRNAs are related to retinoschisin and rhodopsin protein expression. Further fundamental and clinical studies evaluating the role of retinoschisin and rhodopsin in the pathophysiology and diagnosis of DME and other ocular diseases are needed.

We observed that not all biomarkers previously found to be associated with DR<sup>6,9</sup> were detectable in the circulation in our study. We did not find significant amounts of plasma EPC CD133, and RPE 65 mRNA in the circulation of our participants. EPCs express both CD34 and CD133.<sup>21</sup> We observed CD34 mRNA in only 52% of the participants, which may be originating from cell types other than EPCs in the peripheral blood, as CD34 is also expressed by the vascular endothelium.<sup>21</sup> Although the quality of the measurements was confirmed and all measurements were performed in triplicate, it may be possible that the sensitivity of the detection method was insufficient to detect quantifiable amounts of EPCs mRNA and RPE65 mRNA. In addition, this may be associated with differences in expression levels for the markers in patients with DME. Because of this, these markers may not be useful for clinical practice and patients with DME. In contrast, mRNA of retinoschisin and rhodopsin was present in quantifiable amounts in almost all patients with DME, which may improve the chances of clinical utility of these potential biomarkers if these measurements are reproducible among laboratories. It is generally assumed that retinoschisin and rhodopsin are retina-specific proteins that are locally produced.<sup>9,12,13,18–20</sup> This assumption is supported by our finding of higher mRNA levels of retinoschisin in donor retina than in plasma of DME patients. Limitations of this study include the fact that patients had been treated with either bevacizumab or ranibizumab, and our results may have been influenced by that particular anti-VEGF agent. We could not evaluate that because the BRDME study is ongoing and researchers are blinded with regard to the treatment given. Other prospective studies with different anti-VEGF agents and longer follow-up periods should be conducted to further investigate and confirm our findings.

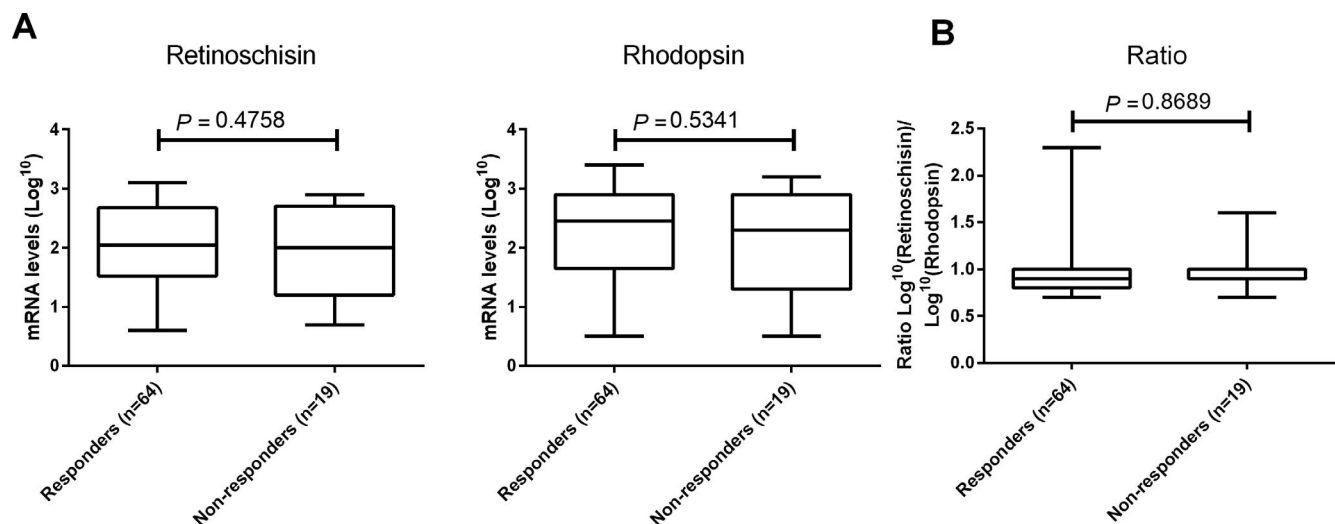


FIGURE 7. Correlation between mRNA levels of retinoschisin and rhodopsin in anti-VEGF responders and nonresponders at baseline and at 6 months. Log10 normalized values of retinoschisin and rhodopsin mRNA (A) and the ratios of retinoschisin to rhodopsin (B) are shown. Responders are defined as patients showing >10% decrease in CST and nonresponders as patients demonstrating ≤10% decrease or increase in central subfield thickness. Boxplots with whiskers from minimum to maximum are shown; central line represents the median. CST, central subfield thickness.

In conclusion, this multicenter, prospective study suggests that retinoschisin and rhodopsin mRNA levels may have value as biomarkers in patients with DME. Further research is needed to establish the relationship between these plasma mRNA levels, corresponding protein levels, and the pathogenesis of DME. Several previous reports have indicated that retina-specific transcripts in the blood, including retinoschisin and rhodopsin, may be promising candidate biomarkers in DR.<sup>9,10,12–14</sup> The identification of retina-specific transcripts in the blood may have implications for a better understanding of disease progression and possibly stratification of patients that may allow the development of more effective treatment strategies in DME, for example, identifying patients most prone to worsening or whose response to therapy is the most significant. Because little is known about biomarkers in DME and because there are no robust methods to determine which patients with DME are good responders and which are nonresponders to anti-VEGF therapy, this study may contribute to the development of more effective treatment strategies of these patients.

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### APPENDIX

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